SUPPLEMENTARY DATA TO:

Structural basis for inhibition of DNA replication by aphidicolin

Andrey G. Baranovskiy[1], Nigar D. Babayeva[1], Yoshiaki Suwa[1], Jianyou Gu[1], Youri I. Pavlov[1,2,3] and Tahir H. Tahirov[1,*]

[1]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198, USA.
[2]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198, USA.
[3]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198, USA.

Running Title: Structure of aphidicolin-Pol ☐ complex

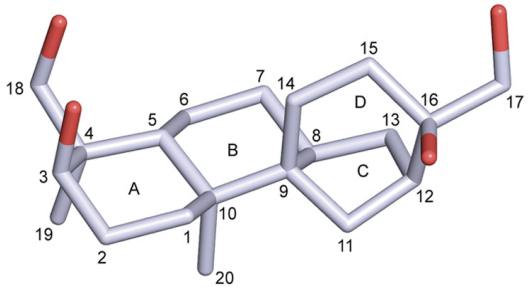
*corresponding author:
Tel: +1 402 559 7607;
Fax: +1 402 559 3739;
Email: ttahirov@unmc.edu

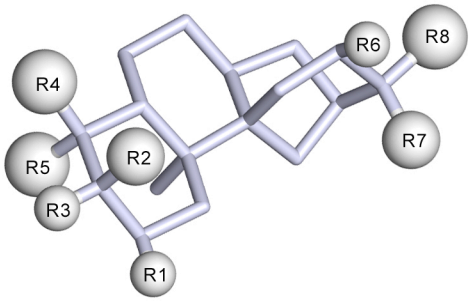
Table S1. X-ray data collection and refinement statistics

	Pol α-DNA/RNA-Aphidicolin
Data collection	
Space group	$P\,2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	105.051, 116.432, 233.026
Resolution (Å)	100.0 - 2.5 (2.54 - 2.50)[a]
[b]R_{merge}	0.071 (0.460)
$I/\sigma I$	26.9 (1.8)
Completeness (%)	97.3 (90.8)
Redundancy	5.0 (3.0)
Refinement	
Resolution (Å)	78.02 - 2.52
No. reflections	93629
R_{work} / R_{free}	23.3 / 26.8
No. atoms	
Protein	13894
DNA	992
Ligand/ion	48
Water	151
B-factors	
Protein	59.3
DNA	62.5
Ligand/ion	34.0
Water	38.1
R.m.s deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.4

[a]Values in parentheses are for the highest-resolution shell.
[b]$R_{merge} = \Sigma_{hkl}\,\Sigma_i\,|I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\,\Sigma_i\,I_i(hkl)$.

Table S2. Inhibitory activity of aphidicolin derivatives[a]





Aphidicolin derivatives	R1	R2	R3	R4	R5	R6	R7	R8	Relative Inhibitory activity (%)
original	H	OH	H	CH2OH	CH3	H	OH	CH2OH	100
3-oxo	H	O		CH2OH	CH3	H	OH	CH2OH	~33[d]
									9[c]
3-deoxy	H	H	H	CH2OH	CH3	H	OH	CH2OH	33[d]
									28[e]
									3.5[c]
3-epi	H	H	OH	CH2OH	CH3	H	OH	CH2OH	<1[b,c,d]
2,3-α-epoxy	-O-		H	CH2OH	CH3	H	OH	CH2OH	2[c]
3α-methyl	H	CH3	H	CH2OH	CH3	H	OH	CH2OH	<1[c]
3β-methyl	H	OH	CH3	CH2OH	CH3	H	OH	CH2OH	1[c]
3β-ethynyl	H	OH	C2H	CH2OH	CH3	H	OH	CH2OH	<1[c]
2-ene-3-deoxy	=		H	CH2OH	CH3	H	OH	CH2OH	<1[c]
2α-methyl	CH3	OH	H	CH2OH	CH3	H	OH	CH2OH	<1[c]
15-ene-16-deoxy	H	OH	H	CH2OH	CH3	=		CH2OH	~33[d]
16-oxo	H	OH	H	CH2OH	CH3	H	O		<1[b,c]
16-demetoxy	H	OH	H	CH2OH	CH3	H	OH	H	<1[c]
17-deoxy	H	OH	H	CH2OH	CH3	H	OH	CH3	4[c]
									<10[d]
17-acetyl	H	OH	H	CH2OH	CH3	H	OH	CH2OAc	20[e,f]
									<1[d]
									10[b,f]
17-glycinate	H	OH	H	CH2OH	CH3	H	OH	CH2OGly	4[c,f]
17-propyl	H	OH	H	CH2OH	CH3	H	OH	n-butyl	<1[c]
17,18-diacetyl	H	OH	H	CH2OAc	CH3	H	OH	CH2OAc	<1[b]
18-deoxy	H	OH	H	CH3	CH3	H	OH	CH2OH	<10[d]
4-demethyl-18-deoxy	H	OH	H	CH3	H	H	OH	CH2OH	<1[c]

Changed groups are shown in red.

[a] Only the derivatives which structures were confirmed by NMR are provided.

[b] (Arabshahi et al., 1988)

[c] (Prasad et al., 1989)

[d] (Hiranuma et al., 1987)

[e] (Haraguchi et al., 1983)

[f] Residual activity might be due to spontaneous deacetylation and/or to contamination by residual aphidicolin.

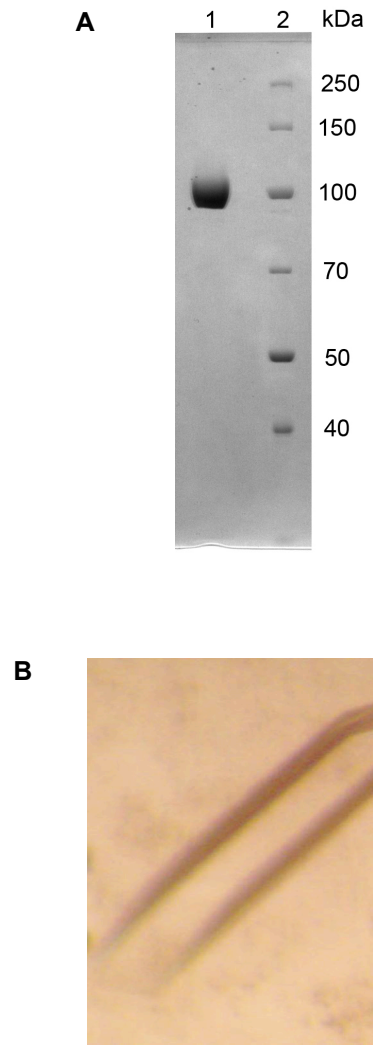


Figure S1. Crystallization of Pol α–DNA/RNA–aphidicolin complex, **(A)** Analysis of the purity of human Pol α catalytic subunit. Lane 1 - p180 core (residues 335-1257); lane 2 - Page Ruler protein ladder (Thermo Scientific). Samples were run on 8% SDS-PAGE and visualized by Coomassie Blue staining. **(B)** Photomicrograph of the Pol α–DNA/RNA–aphidicolin crystal.

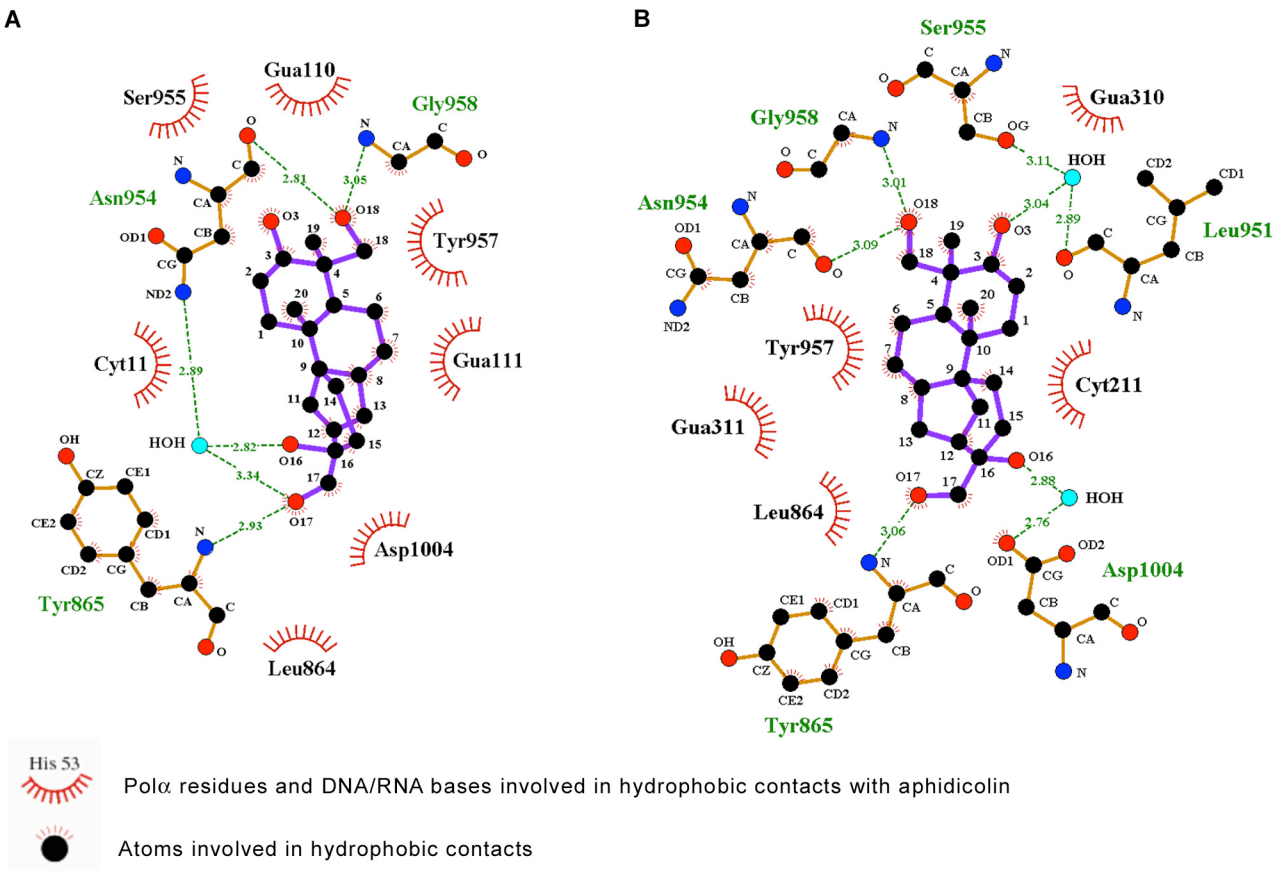


Figure S2. Schematic representation of the aphidicolin contacts in the first **(A)** and in the second **(B)** independent molecules of the asymmetric unit in the crystal. The hydrogen bonds are shown by green dashed lines. The hydrogen bond between 18-hydroxy group of aphidicolin and a main-chain oxygen of Asn954 has not been automatically recognized by LigPlot+ and added manually according to software instructions.

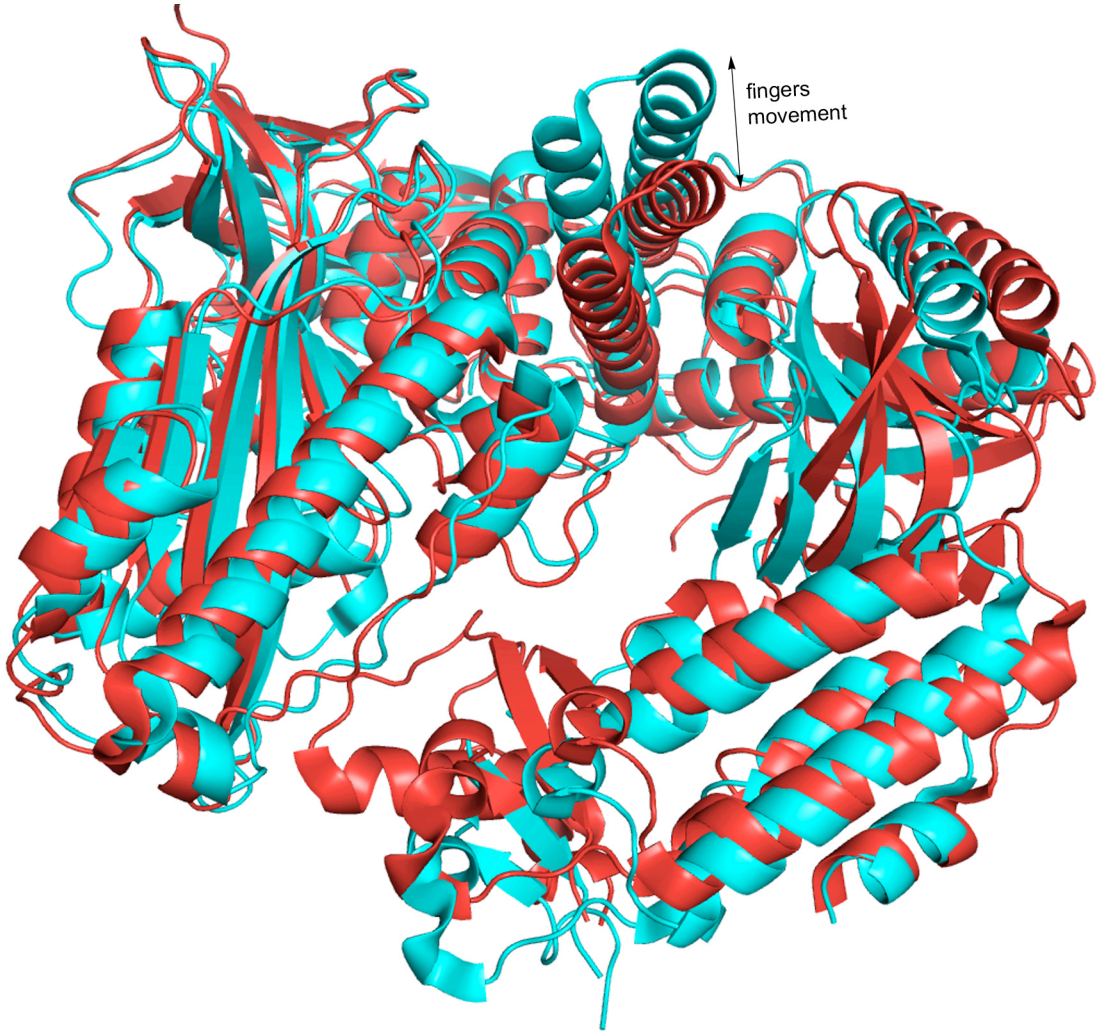


Figure S3. Alignment of the yeast Pol α in the closed and open forms (PDB codes 4FYD and 4FXD, respectively). Protein is represented as cartoon and colored red or cyan in the closed or open conformation, respectively. DNA, RNA and dGTP molecules were omitted for clarity.

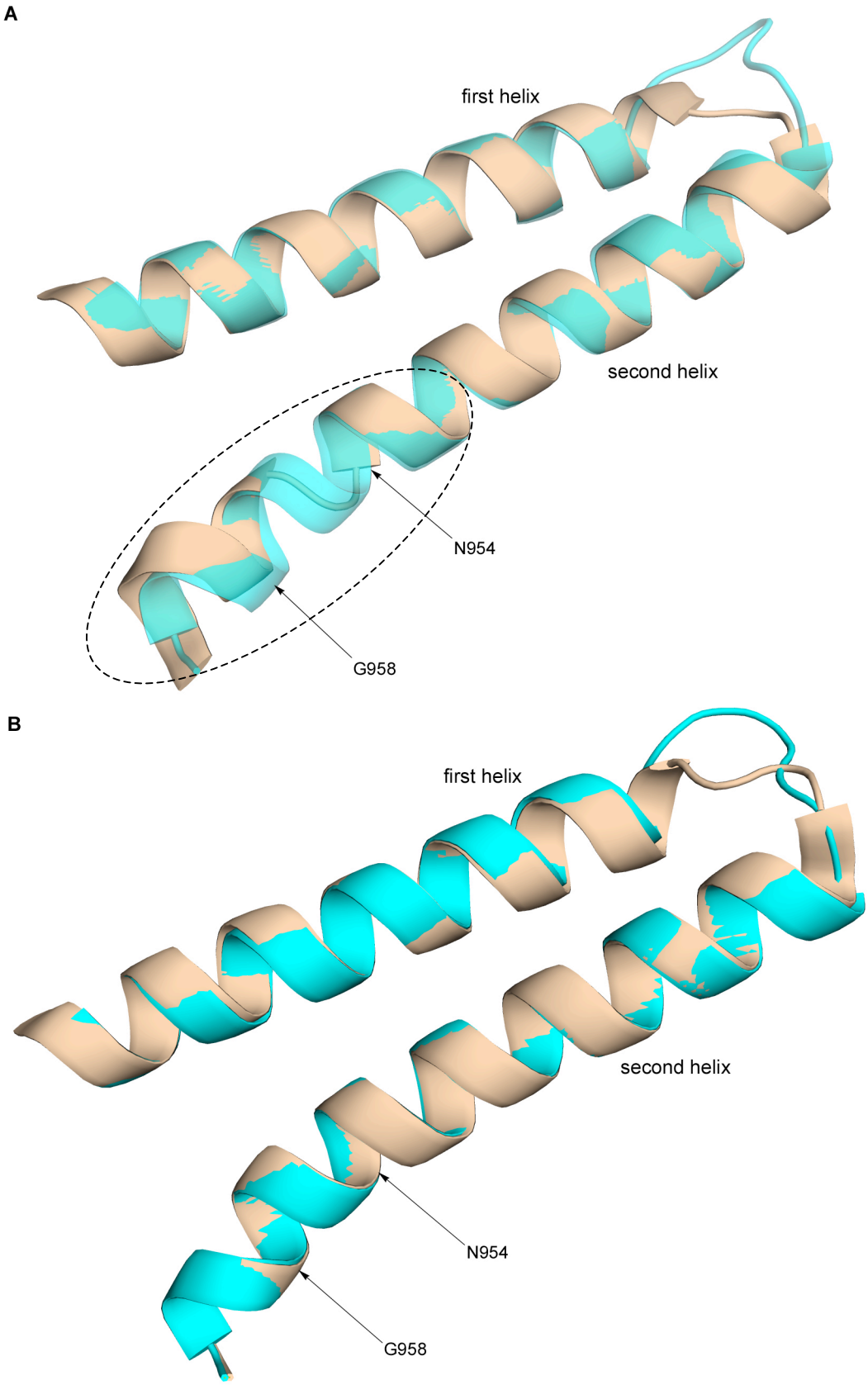


Figure S4. Alignment of the fingers domain from the human and yeast Pol α in the open **(A)** and closed **(B)** forms. Fingers of the human Pol α in complex with DNA/RNA and aphidicolin **(A)** or dCTP **(B)** are colored cyan, from the yeast Pol α in complex with DNA/RNA **(A)** or DNA/RNA–dGTP **(B)** – wheat (PDB codes 4Q5V, 4QCL, 4FXD, and 4FYD, respectively). The cartoon for Pol α–DNA/RNA–aphidicolin complex is shown with 40% transparency. Positions of the residues in human Pol α interacting with aphidicolin are shown by arrows. The circled helix region was shown in more details on Figure 4B.

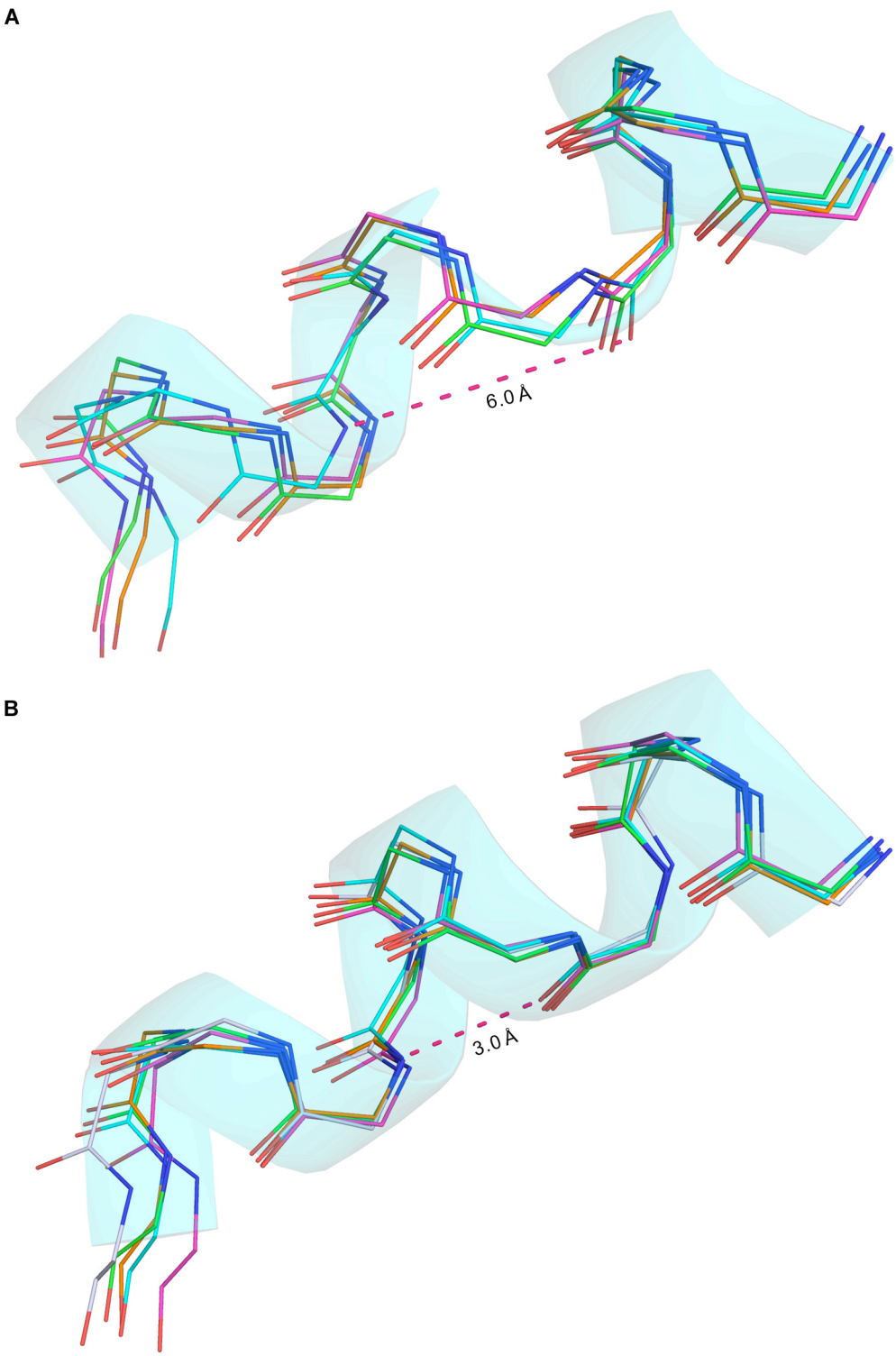


Figure S5. Close-up view of the second helix distortion in B-family DNA polymerases in the open **(A)** and closed **(B)** forms. Second helices from different polymerases were aligned with that one from the yeast Pol α represented as cartoon with 80% transparency. **(A)** The carbon is colored cyan for the yeast Pol α, green – for the bacteriophage RB69 DNA Pol, orange – for the *E.coli* DNA Pol II, and magenta – for the Herpes Simplex virus type 1 DNA Pol (PDB codes 4FVM, 1IH7, 3K5O, and 2GV9, respectively). **(B)** The carbon is colored cyan for the yeast Pol α, green – for the bacteriophage RB69 DNA Pol, orange – for the *E.coli* DNA Pol II, magenta – for the yeast Pol δ, and grey – for the yeast Pol ε (PDB codes 4FYD, 1IG9, 3MAQ, 3IAY, and 4M8O, respectively). The distance between Gly952 N and Asn948 O in the yeast Pol α is indicated (analogs of Gly958 and Asn954 in the human Pol α, Figure 4B).

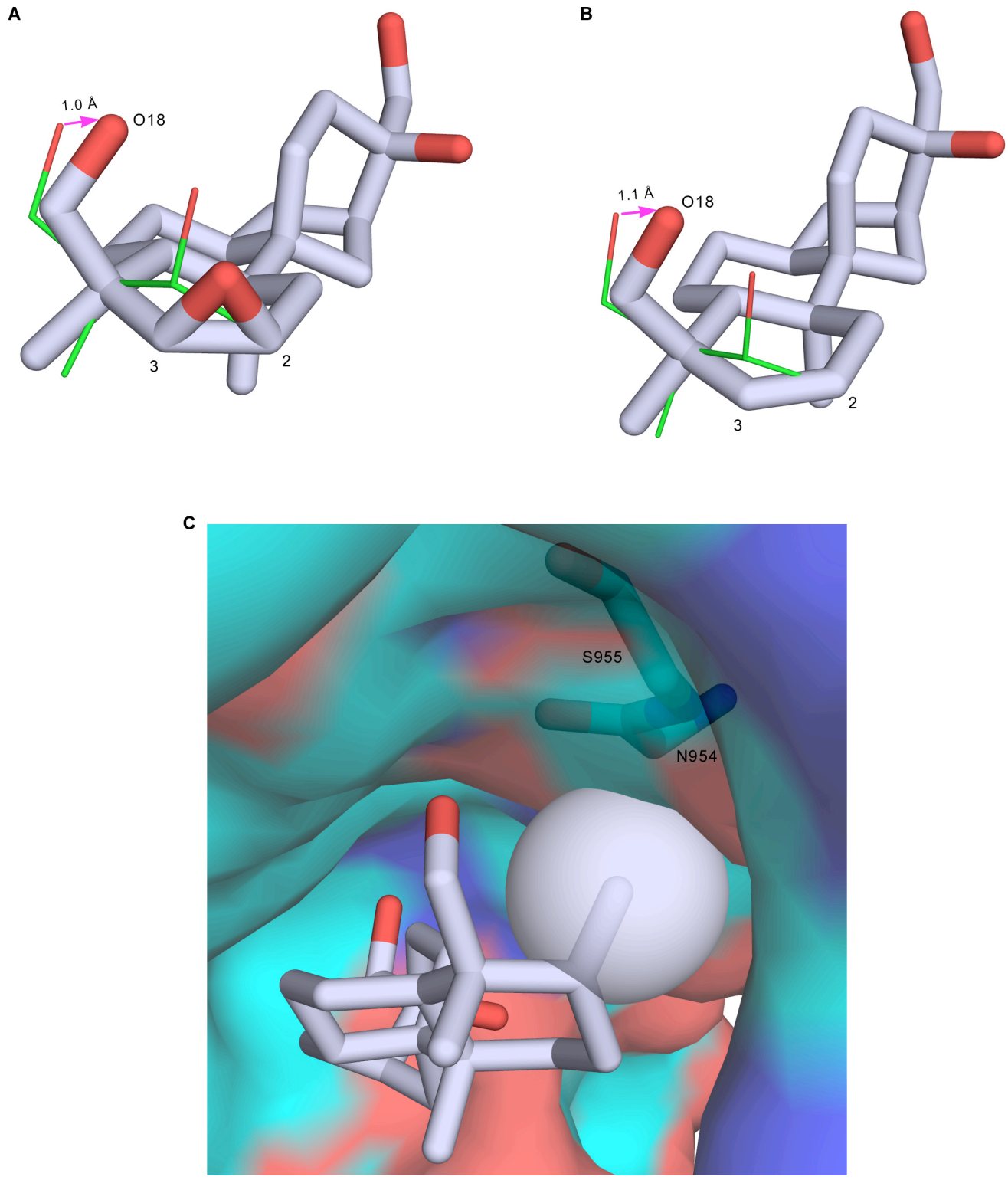


Figure S6. Modelling of aphidicolin derivatives 2,3-α-epoxy **(A)**, 2-ene-3-deoxy **(B),** and 3α-methyl **(C).** The representation of aphidicolin and its derivatives is the same as on Figure 6. Magenta arrows show the shift of O18. **(C)** The protein is represented as surface with 20% transparency, and colored cyan for carbon, red for oxygen, and blue for nitrogen. 3α-methyl is shown as sphere with 20% transparency. The main-chain atoms of Asn954 and Ser955 are shown as sticks.

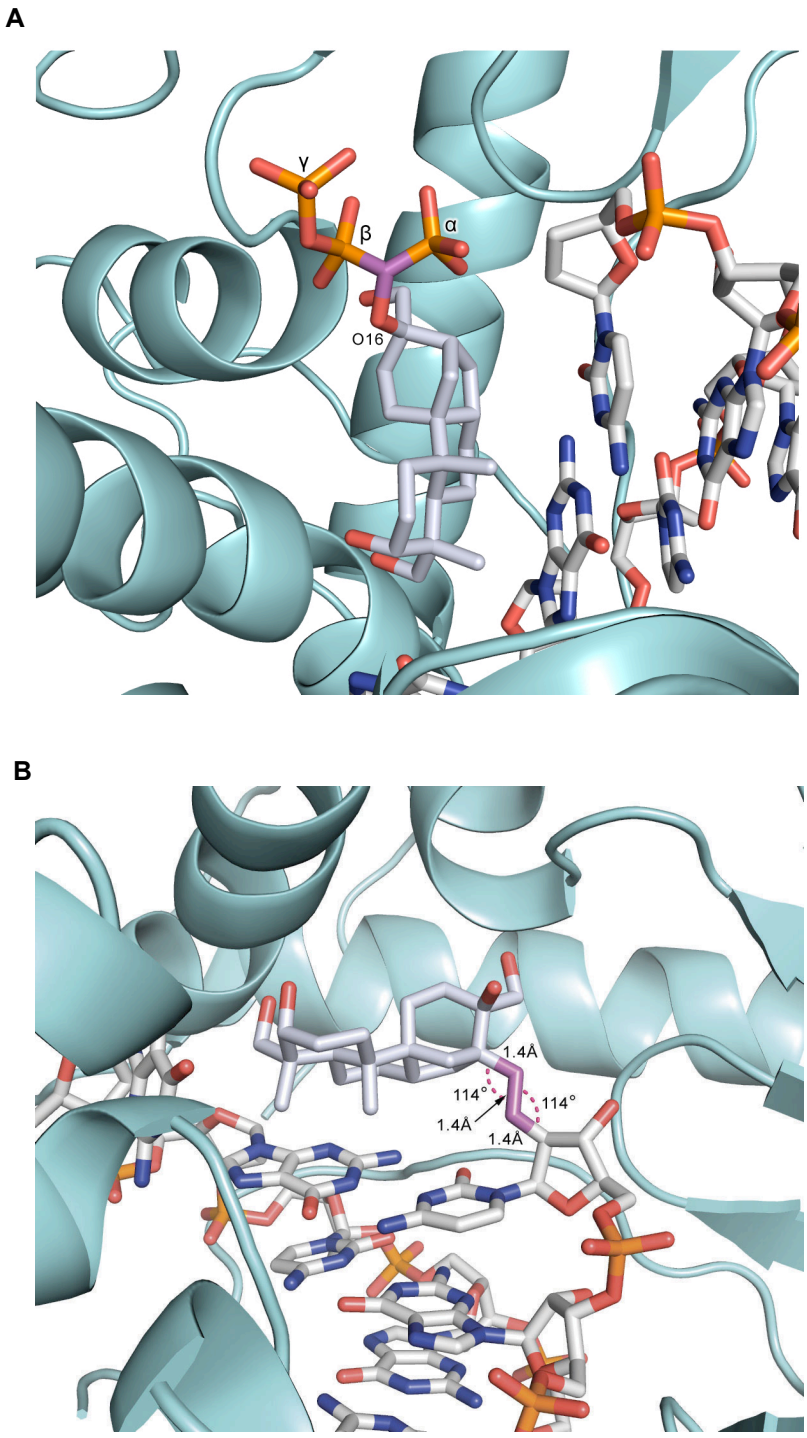


Figure S7. Design of new aphidicolin derivatives. The protein from the complex with aphidicolin is represented as cartoon and colored cyan; the color scheme for DNA/RNA and aphidicolin is the same as on Figure 3B. **(A)** Aphidicolin with triphosphate group attached to O16. The atom of triphosphate bridging it with O16 of aphidicolin is colored magenta. For modelling we used the aligned structures of Pol α-DNA/RNA ternary complexes containing aphidicolin or dCTP (Figure 3B). **(B)** Aphidicolin connected with the 2'-end of RNA strand through C12. Atoms of the linker connecting aphidicolin and 2'-end of primer are colored magenta. The lengths of covalent bonds between linker atoms and their neighbors are all 1.4 Å.